SYNTHESIS, CHARACTERIZATION, AND CYCLIC STRESS-INFLUENCED DEGRADATION OF A POLY(ETHYLENE) GLYCOL BASED POLY(BETA-AMINO ESTER)

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SYNTHESIS, CHARACTERIZATION, AND CYCLIC STRESS-INFLUENCED DEGRADATION OF A POLY(ETHYLENE) GLYCOL BASED POLY(BETA-AMINO ESTER)

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SUMMARY

Poly(beta-amino esters) are photopolymerizable and biodegradable polymers prepared by the combination of amines with diacrylates. This study aims to fundamentally understand the polymer network formed by poly(ethylene)glycol diacrylate (PEGDA) MW 700 and 3-methoxypropylamine (3MOPA) as well as to characterize the degradation response of this material with and without cyclic loading. The networks were formed by a two-step process; (1) the synthesis of amine-co-peg diacrylate macromers through a step growth reaction, followed by (2) UV initiated chain growth network formation of the diacrylated macromers. Macromer reaction chemistry was confirmed by ^{1}H NMR measurements. UV calorimetric analysis revealed that network formation was dependent on molecular weight of the PEGDA monomer and light intensity, but not temperature in the range of 20 °C to 40 °C. The glass transition temperature of all networks was measured to be in the range of -40 °C to -30°C with a rubbery moduli ranging from 4 to 10 MPa, depending on the molecular weight of the PEGDA monomer. Partial crystallization was discovered to occur in the networks containing higher molecular weight PEGDA only in the presence of humidity and high frequency cyclic loading. Degradation studies were performed with and without applied cyclical stress, and in both cases elastic modulus decrease and mass loss occurred steadily over a 24-hour period. Increasing frequency of applied compressive stress during degradation served to slightly lower degradation rates, especially in samples cycled at high frequency, which crystallized. In all materials, applied cyclic load resulted in catastrophic fracture of the material prior to an appreciable decrease in modulus. The experiments reveal that degradation rate and failure mode can be influenced by the

addition of cyclic loading and this should be considered when screening biodegradable polymers for applications that include mechanical loading.

CHAPTER 1

INTRODUCTION

Degradable polymers have attracted significant attention in the field of biomaterials, and advances in polymer synthesis have allowed for the development of biopolymers with a wide range of mechanical properties. Applications of degradable biopolymer materials range from drug delivery to orthopedic devices.[1] The appeal of these materials is their ability to degrade safely and be resorbed into the body circumventing surgical removal or concern over chronic toxicity or carcinogenicity effects.[2] In addition, degradation can also be used to deliver therapeutic drugs to promote biological response or to facilitate tissue in-growth in tissue engineering applications. Although biodegradable materials have significant promise, there are some practical drawbacks of biodegradable materials currently used in FDA approved applications, particularly in regards to synthesis and independent adjustability of degradation and mechanical properties. A familiar and widely used degradable biomaterial, PLGA requires ring opening polymerization of L-lactide and glycolide, plus purification steps,[3,4]which results in by-products, increased production time and material cost. Unfortunately, complex polymerization and additional purification steps are required for many other biopolymers as well, especially those with multifunctional macromers.[5-11] In addition, PLGA polymers are typically glassy at body temperature, and independent adjustability in degradation rate and critical mechanical properties such as elastic modulus and toughness has not been demonstrated. In this vein, researchers have produced several alternatives to PLGA polymers, although examination of the structure and mechanical performance of these materials is in its infancy.

There is considerable recent interest in a class of degradable polymers know as poly(beta amino esters) synthesized by Langer and colleagues. The macromer precursors to the polymer networks are synthesized by a condensation reaction between

commercially available diacrylates and amines, which produces no by-products. Furthermore, these polymers are photocrosslinkable and photo-initiator can be mixed directly into the macromer without the use of solvents. The simplicity of the two step reaction, and the commercial availability of a vast array of amine and diacrylate monomers, leads to a wide range of possible chemistries with tailored degradation rates, potentially safe products, and tunable mechanical properties[5]. These benefits, plus the ease of fabrication afforded to these polymers through photocrosslinking of the network has rendered them of great interest for applications in drug-delivery, tissue engineering, and microdevices.[5] We believe the poly (beta amino esters) system has considerable promise beyond these initial applications and further understanding of the network structure and mechanical properties would be beneficial to advancing the future use of this material.

Prior work has examined the elastic modulus and degradation rates of a broad range of poly(beta amino esters)[5]. In the present work we focus on a poly(beta amino esters) formed from a combination of poly(ethylene glycol) diacrylate (PEGDA) and 3-methoxypropylamine (3MOPA). Emphasis is placed on understanding the network formation under UV irradiation and the subsequent network breakdown in saline solution. Given the importance of applied load in some biomedical applications of biodegradable polymers[12,13], we characterize the degradation response with and without applied cyclic loading.

CHAPTER 2

MATERIALS AND METHODS

Synthesis and Reaction Characterization

Macromer and Sample Preparation

During the course of this study we examined a specific class of degradable photocrosslinking polymers known as poly(beta-amino esters), which are formed by the combination of diacrylates and primary or secondary amines[5,14-18]. Our focus was the combination of poly(ethylene glycol) diacrylate (PEGDA) and 3-methoxypropylamine (3MOPA). For this study, PEGDA monomers (with molecular weights of 700, 575, and 258) and 3MOPA were purchased from the Sigma-Aldrich chemical company. The PEGDA monomers were mixed with 3MOPA and reacted by the previously established method of mixing the monomers for 24 hours at 90˚C to form linear macromers with an ester/(tertiary amine) backbone[5]. The optimal acrylate to amine molar ratio of 1.2, shown to maintain the acrylate chain ends and promote a crosslinked network, was maintained for all reactions[5]. The result of this process yielded three macromer mixtures capable of forming the desired crosslinked networks. The photo-initiator, dimethoxy-2-phenyl-acetophenone purchased from Sigma Aldrich, was dissolved directly into the macromer mixtures through ambient temperature stirring. These mixtures either were placed between two glass slides by pipette to produce samples which were approximately 1 mm thick for use in thermo-mechanical analysis, or poured into molds producing cylindrical samples which measured approximately 24.5 mm in diameter and 12 mm in height for compression and degradation studies. The filled molds were placed under a UV lamp, capable of delivering approximately 9.0 mW/cm2 at a distance of 10 inches from the sample, to form the crosslinked network. Formation of the network in the 1mm thick samples took approximately 5 minutes (confirmed by PhotoDSC experiments), whereas

the larger compression samples were exposed to the UV light for 30 minutes to ensure complete polymerization.

Hydrogen-1 Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR)

The aforementioned reaction of PEGDA MW 700 and 3MOPA to produce PEG-MOPA diacrylate macromer precursors for each mechanical study was carried out over a 24-hour period and is illustrated in Figure 1. The step growth reaction is straightforward; with the addition of heat and the mixing of the monomers in the correct ratio, the PEGDA monomers react with the primary amine groups of the 3MOPA to form the macromer precursor to our network. To characterize this reaction, ^{1}H NMR analysis was performed on the macromer mixture of PEGDA MW 700 and 3MOPA at various stages during the reaction. For comparison purposes, samples of PEGDA MW 700 and 3MOPA were first individually tested using ^{1}H NMR followed by testing the samples of the macromer mixtures after 6 hours, 11 hours, 16 hours and 24 hours reaction time.

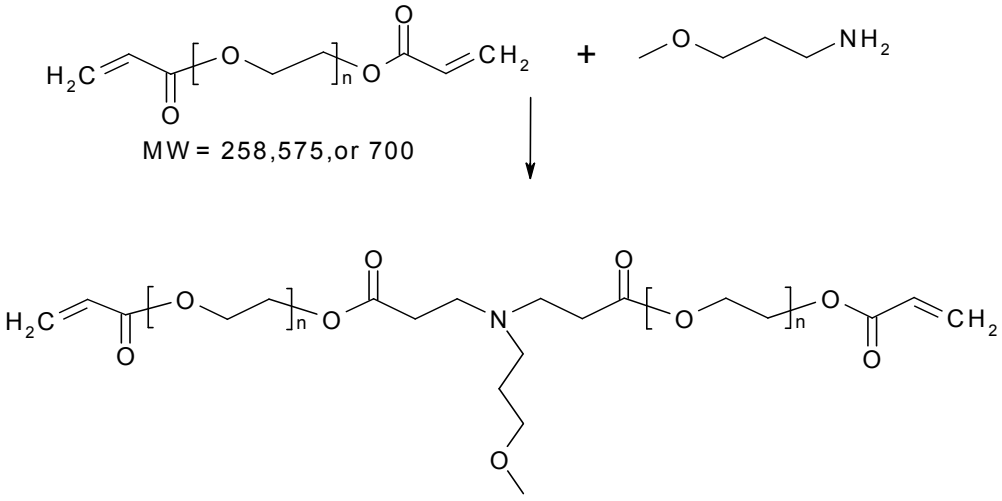


Figure 1. Reaction sketch of PEGDA combining with 3MOPA to form the macromer mixture.

Photo Differential Scanning Calorimetry (PhotoDSC)

The network formation form the macromers in Figure 1 was characterized for PEGDA molecular weights of 258, 575, and 700 at two temperatures and two UV intensities using PhotoDSC. The PhotoDSC experiments were performed using a TA Instruments Q20 DSC with a photocalorimetry accessory (PCA) setup. The PCA allows UV/Visible light from a 100W high pressure mercury source to be conveyed to the sample chamber of the DSC via dual light guides. For each PhotoDSC experiment, small macromer samples of the different fully reacted PEGDA with 3MOPA mixtures were weighted into aluminum DSC pans and placed in the DSC sample chamber. One light guide was placed over the sample and one light guide over a reference site. The chamber was allowed to equilibrate for two minutes at a specified temperature before the shutter on the PCA was opened, exposing the samples to UV light. After the PCA shutter was opened, measurements of heat flow into the sample were taken for eight minutes as the macromer sample formed the crosslinked network. The heat flows versus time curves obtained by PhotoDSC were used to calculate percent conversion of the sample from macromer to networked polymer. Complete conversion was assumed to occur at the time when the heat flow into the sample reached a steady rate, and conversion was calculated as the fraction of complete conversion as a function of time. PhotoDSC experiments were carried out at two different temperatures, 20˚C and 40˚C, and two different intensities, 5mW and 8mW, in combination. Conditions were chosen to mimic extreme conditions associated with the lab top UV lamp setup used in polymerizing samples for mechanical testing. Three samples of similar weights of each macromer were used for each combination of conditions. Percent conversions obtained over the time period of conversion were then averaged over the three runs.

Materials Properties Characterization

Dynamic Mechanical Analysis (DMA)

Samples of the polymer mixtures were characterized thermally by DMA and DSC and the mechanical behavior of PEGDA MW 700 with 3MOPA was characterized using the MTS Insight™ mechanical tester. DMA experiments were conducted using a tensile clamp and a 1 mm thick sample. Modulus versus temperature curves were obtained from DMA experiments using a 0.1% strain amplitude and a -100˚C to 100˚C temperature range. Testing was preformed on three samples of each PEGDA/3MOPA combination and the results for glass transitions (T_g), modulus, and crosslink density were obtained and averaged over those three samples. T_g and modulus of the materials were obtained from the graphs of storage modulus vs. temperature curves, whereas crosslink density values were calculated from the rubbery modulus. After the initial testing to characterize the PEGDA with 3MOPA combinations, the combination of PEGDA MW 700 with 3MOPA was tested over two different temperature ranges, -80˚C to 100˚C and -60˚C and 100˚C, to verify the occurrence of a post-T_g secondary transition revealed as a small peak in storage modulus. From testing the nine samples of PEGDA700 with 3MOPA over three temperature ranges on the DMA, it was determined that further exploration of the PEGDA MW 700/3MOPA material should be performed using the DSC since the secondary transition was present in all cases.

Differential Scanning Calorimetry (DSC)

DSC experiments were performed on samples of all polymer networks to screen for any additional reactions not detected by DMA. DSC was preformed on three samples of each material. The method used to generate the heat flow versus temperature curves from the DSC entailed first heating the sample to 300˚C followed by cooling to -100˚C and heating again to 300˚C. For these materials, transitions aside from glass transition

were not observed in undeformed samples. Further DSC testing was conducted on the PEGDA MW 700/3MOPA samples based on the secondary transition observed in DMA results. DSC by the same heat-cool-heat method was performed on samples of PEGDA MW 700 with 3MOPA which had undergone the various treatments. Prior to DSC testing sample of PEGDA MW 700/3MOPA were either; (1) strained for 12 hours or more, (2) strained and then submerged in water for 12 hours or (3) exposed to cyclic loading while being submerged in water for 24 hours. All samples which were dried for 48 hours in a vacuum oven prior to DSC testing.

Degradation Behavior

It was previously established that the class of poly(beta-amino esters) degrade by hydrolysis into the following degradation products: bis-beta-amino acids, diols, and poly(acrylic acid) chains[5]. In order to better understand the degradation of this material, we studied its degradation with and without applied cyclic load in phosphate buffered saline (PBS) solutions. To create a baseline for the material's degradation, we tracked weight loss and elastic modulus as a function of fluid exposure without applied load. We first weighed and then placed a number of duplicate samples in individual vessels filled with PBS solution pre-heated to 37˚C. The vessels containing the samples were placed into an oven which maintained the temperature for 24 hours. Every two hours, three to five samples were removed and weighed. After materials had been removed from the oven and weighed they were dried for 48hrs at room temperature. The dried materials were tested by compression on and MTS Insight™ to obtain the elastic modulus at small strain.

In situ Cyclic Loading

Cyclic compression testing was preformed on the polymer samples using the MTS Insight™ with the addition of the environmental attachment containing water at

37˚C. The use of PBS was prohibited by this setup due to the composition of the compression platens; therefore, distilled water was used in cyclic testing. During these experiments the Insight™ repeatedly compressed the sample soaking in water to a 10% amplitude at a strain rate of 0.1/s during the loading cycle. The frequency of applied compressive cycling was varied from 5.56×10^{-4} Hz to 1 Hz to observe the contribution of the frequency of the compressive loading cycle to the materials' degradation profile. These various testing frequencies corresponded to intermittent cycle times of 2 hours, 1 hour, 15 minutes, 5 minutes or 1 second between each compression cycle.

CHAPTER 3

EXPERIMENTAL RESULTS

Synthesis of PEGDA varied MW and 3MOPA Networks

^{1}H NMR

Since our major focus for subsequent mechanical testing was on the combination of PEGDA MW 700 with 3MOPA, we performed ^{1}H NMR testing on the macromer formation involving these materials only. ^{1}H NMR was performed at various times during the reaction in order to gain a clear understanding of the reaction. Figures 2-7 show the ^{1}H NMR results. In Figures 2, 3 and 4 the peaks discussed in the tables are represented by letters a-f, g-k and a-m, respectively. A sketch of the molecule represented in each NMR spectra is provided and labeled to indicate the hydrogen in the molecule and the peaks in the spectra corresponding to the respective hydrogen. The ^{1}H NMR results of the base materials, PEGDA MW 700 seen in Figure 2 and 3MOPA seen in Figure 3, show the initial placement of the peaks. When these spectra are compared to Figure 4, which represents these materials after they have reacted for 24 hours, we see the end result of the macromer formation. Figure 4 shows the anticipated disappearance of one peak, k from the amine group (Figure 3), and the formation of two peaks, l and m. Peaks l and m correspond to new bonds between the amine and PEG in the macromer. The acrylate groups shown by peaks a and b are still present in the reacted mixture (Figure 3) as expected for an acrylate rich solution resulting in diacrylated macromers.

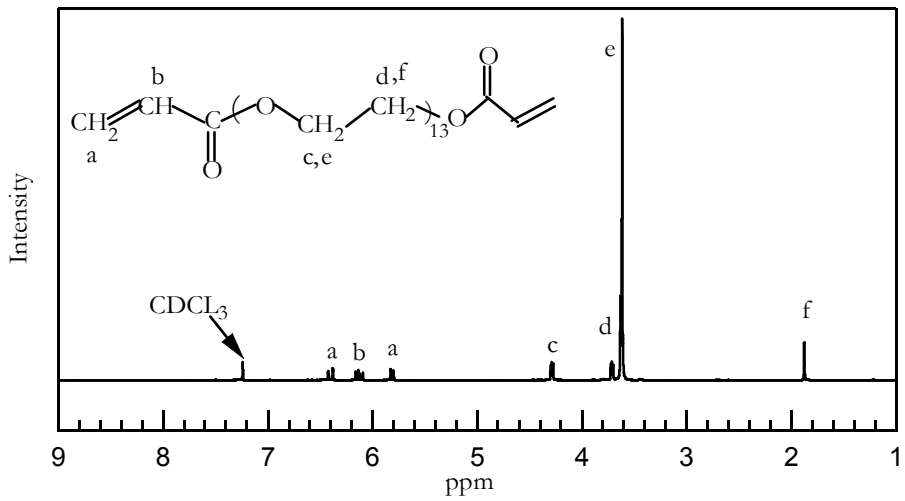


Figure 2.[1]H NMR results for PEGDA700 with sketch of molecule and labeled hydrogens corresponding to labeled peaks.

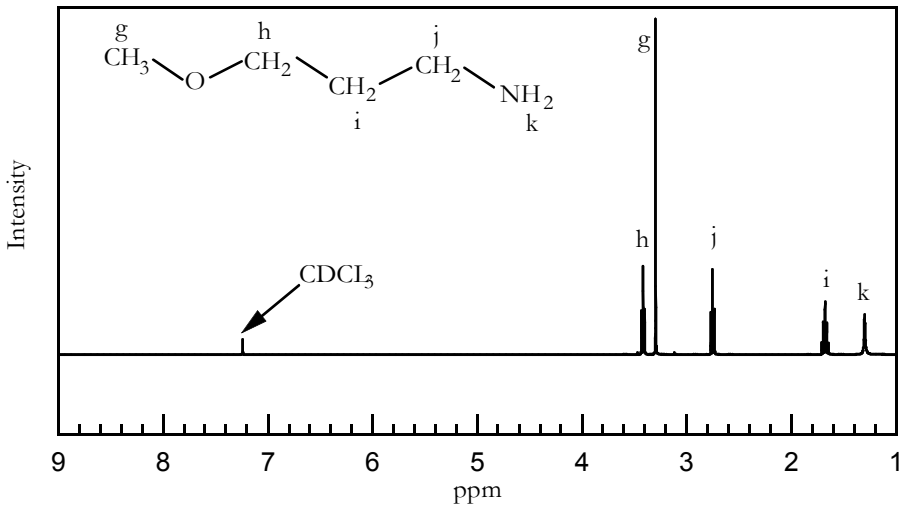


Figure 3.[1]H NMR results for 3MOPA with sketch of molecule and labeled hydrogens corresponding to labeled peaks.

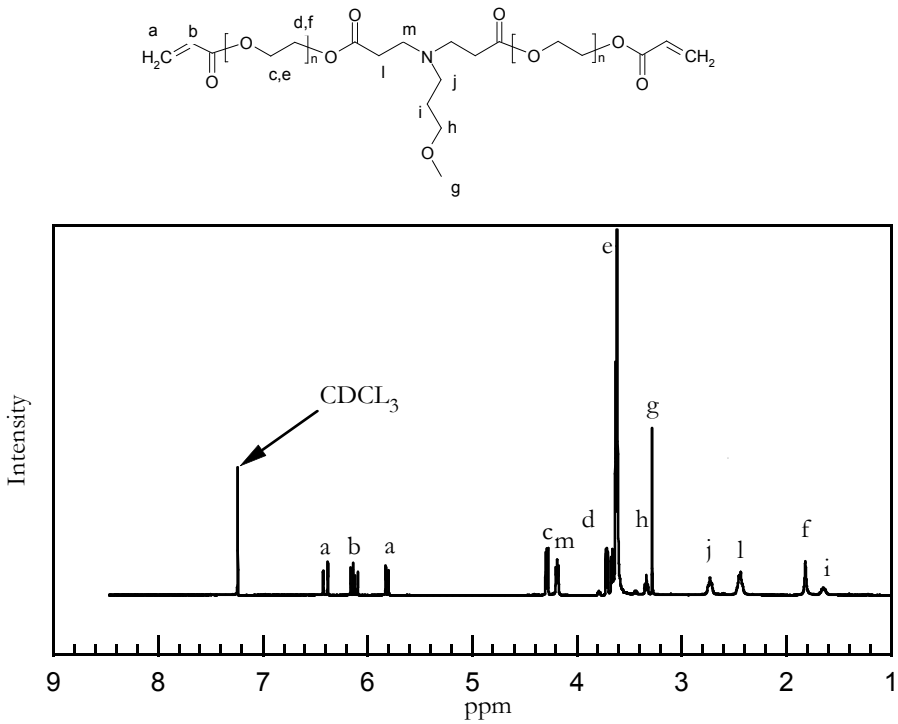


Figure 4. ^{1}H NMR results for PEGDA700/3MOPA mixed for 24 hours with sketch of molecule and labeled hydrogens corresponding to labeled peaks.

The results of the ^{1}H NMR data for the varied time periods of 6, 11, 18, and 24 hours at areas of interest in the spectra are shown in Figures 5-7. It can be seen in Figures 5 that the disappearance of the peak, k at 1.3 ppm, which was previously seen in Figure 3 on the amine head, occurs within the first 6 hours of mixing. This peak represents the primary amine group and its disappearance is in compliance with the reaction sketch in Figure 1. Also occurring in Figure 5 is the flattening of the peak at 1.7 ppm, referred to as i, primarily due to the significance of the hydrogen atoms attached to the amine being out weighed by the high concentration of the PEGDA. We also noted the disappearance and reappearance of peak referred to as e at 1.88 ppm it is our believe that the appearance of peak e is not only related to the length of the PEG repeat unit in the diacrylate monomer, but is also dependant on symmetry of the end groups attached to the repeat units. The two peaks important to macromer formation, l and m, can be observed in Figure 6 and 7 and they occur in the first 6 hours.

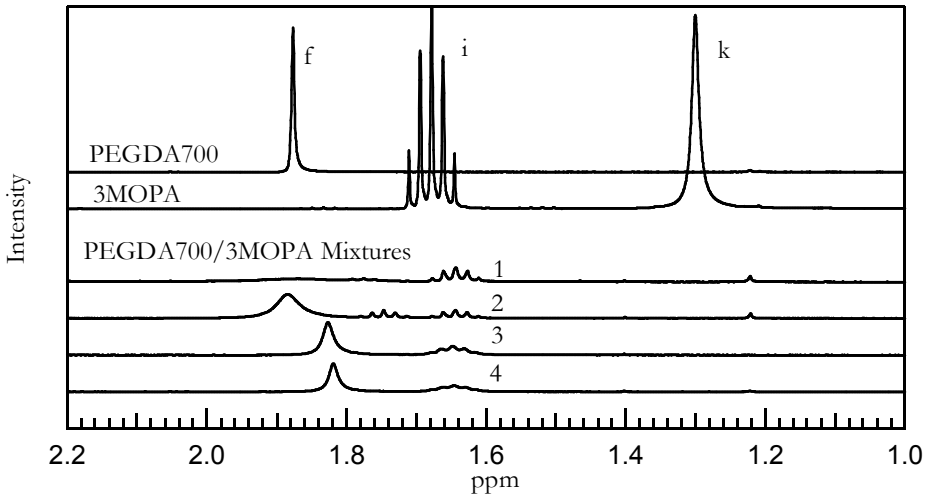


Figure 5. ^{1}H NMR results for PEGDA 700, 3MOPA and PEGDA700/3MOPA mixed for 6 hours, 11 hours, 16 hours and 24 hours from 2.2-1.0 ppm.

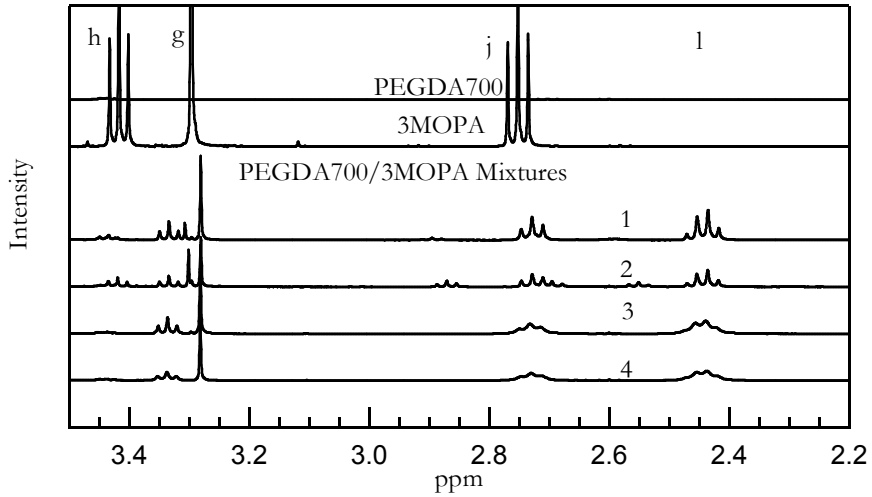


Figure 6.^{1}H NMR results for PEGDA 700, 3MOPA and PEGDA700/3MOPA mixed for 6 hours, 11 hours, 16 hours and 24 hours from 3.5-2.2 ppm.

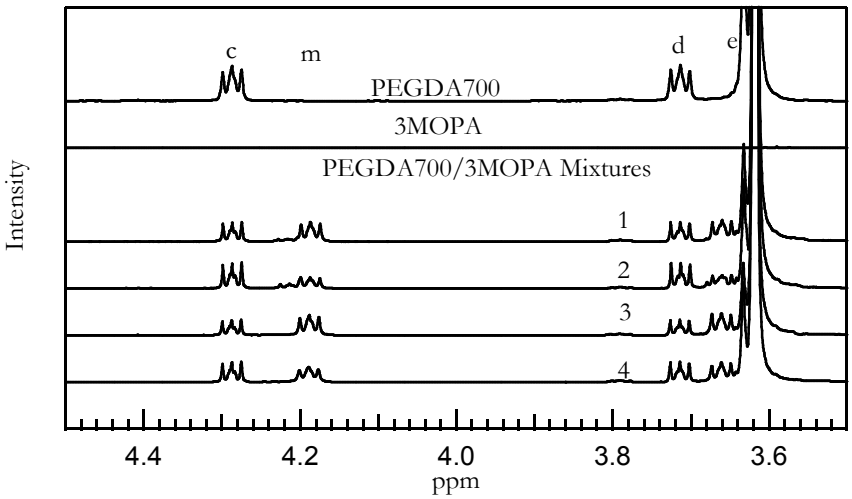
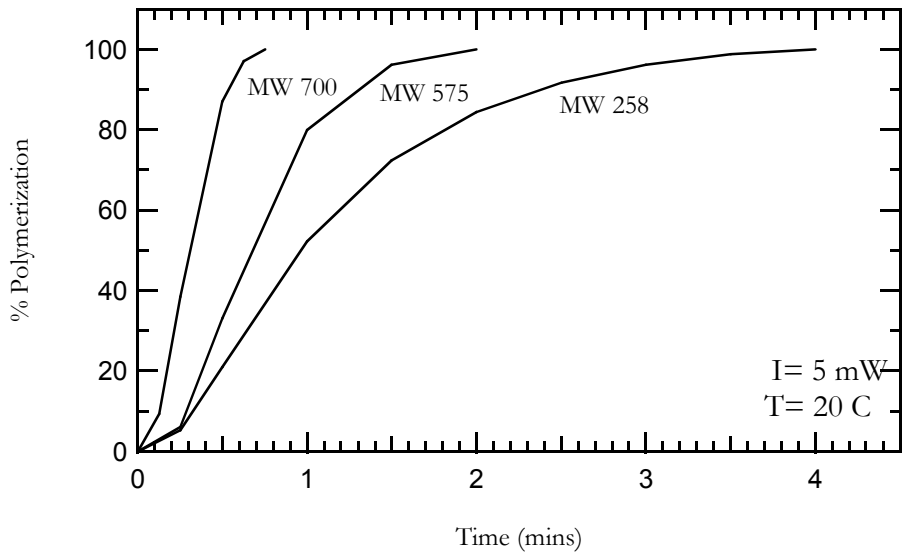


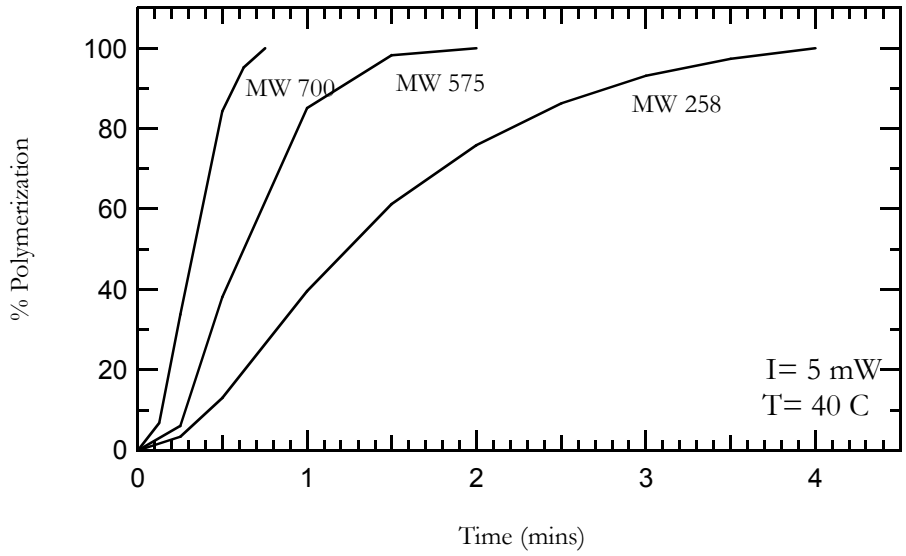
Figure 7. ^{1}H NMR results for PEGDA 700, 3MOPA and PEGDA700/3MOPA mixed for 6 hours, 11 hours, 16 hours and 24 hours from 4.5- 3.5 ppm.

PhotoDSC

PhotoDSC was used to establish the conversion rate of the macromer solutions into a polymer network for various conditions. Figure 8 depicts the results obtained from the PhotoDSC experiments. For these experiments we chose two intensities, 5mW/cm^2 and 8mW/cm^2, and two temperatures, 20˚C and 40˚C to represent the range in practical experimental conditions. Figures 8 a, b, c, and d show the normalized percent conversion of the networked polymer for combinations of PEGDA at various molecular weight and 3MOPA at 5mW and 20˚C, 5mW and 40C, 8mW and 20C, and 8mW and 40C respectively. In comparing Figures 8a to 8b and 8c to 8d, a 20 °C increase in temperature had a negligible impact on network formation kinetics. When comparing Figures 8a to 8c and 8b to 8d light intensity had a small effect on the conversion rate of the networks, and this effect depended on the molecular weight of the PEG monomer. The factor having the largest influence on conversion was found to be the molecular weight of the PEG unit in the diacrylate monomer. Conversion time decreased for longer PEGDA monomer and the smallest PEGDA monomers were the most sensitive to light intensity.

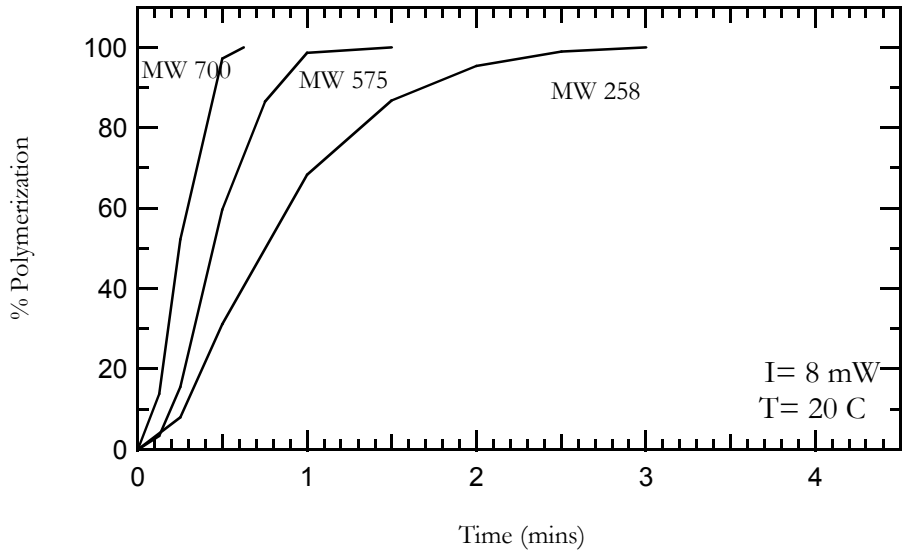


(a)

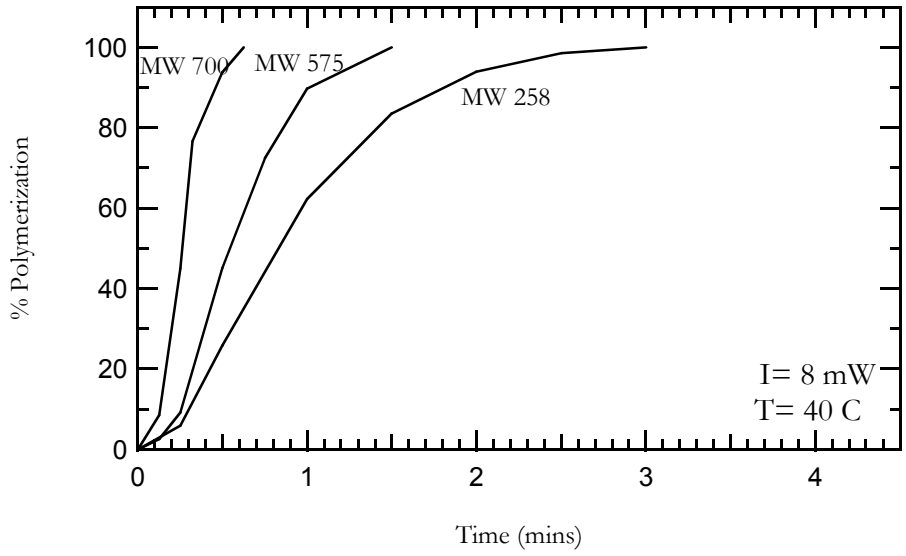


(b)

Figure 8. Rate of polymerization by photo DSC for PEGDA700, PEGDA575 and PEGDA 258 mixed with 3MOPA at (a) 5mW and 20°C, (b) 5mW and 40°C (c) 8mW and 20°C, (d) 8mW and 40°C.



(c)



(d)

Figure 8. Continued.

Basic Characterization of PEGDA with 3MOPA Networks

DMA

Thermo-mechanical experiments were carried out to characterize the network formed by the reaction of the diacrylated macromers. DMA was performed on duplicate samples of various combinations. The glass transition temperatures, modulus, and crosslink density at 25˚C obtained as a result of the DMA experiments were averaged over all samples. The data is presented in Tables 1 and 2. A graph of storage modulus versus temperature for a representative sample of each PEGDA molecular weight combination with 3MOPA is presented in Figure 9. PEGDA MW 700 with 3MOPA had the lowest glass transition temperature at -39˚C followed by PEGDA MW 258 with 3MOPA at -35˚C and PEGDA MW 575 with 3MOPA had the highest at -31˚C. Modulus of the materials and crosslink density at 25˚C were the highest for mixtures of PEGDA MW 575 followed by mixtures of PEGDA MW 700 and the lowest for mixtures of PEGDA MW 258. In all of the PEGDA MW 700 with 3MOPA DMA experiments, a storage modulus "peak" was observed between -15˚C and 15˚C (Figure 9). It is believed that this peak is related to the crystallization behavior this network exhibits when it is strained cyclically at high frequency and exposed to water. This hypothesis was verified using DSC results for several strained and saturated samples of the material.

Table 1. Glass Transition Temperature and Modulus Data for Samples of Networked PEGDA of Various MW with 3MOPA.

MW of PEGDA	# of Samples	Average Tg (˚C)	Standard Deviation from Tg	Average Modulus at 25˚C (Mpa)	Standard Deviation from Modulus at 25˚C
700	3	-39.63	0.33	4.663	0.356
575	3	-31.51	1.33	8.394	1.077
258	3	-35.07	0.54	3.011	0.689

Table 2. Crosslink Density of Networked Samples of PEGDA of Various MW with 3MOPA.

MW of PEGDA	# of Samples	Average Stress at 25˚C (dyne/cm^2)	Average Extension ratio at 25˚C	Average Crosslink Denstiy at 25˚C (mol/cm^3)
700	3	46603	1.001	2.55×10^{-4}
575	3	83610	1.008	3.05×10^{-4}
258	3	28796	1.033	2.41×10^{-5}

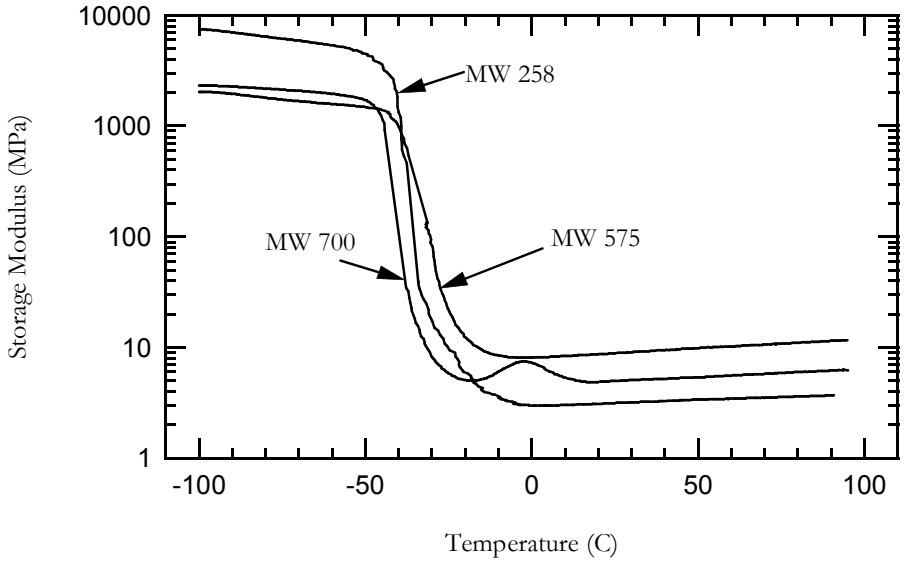


Figure 9. DMA Results for Networked Sample of Various MW PEGDA and 3MOPA.

DSC

Figure 10 shows the DSC curves for all of the virgin materials; none of the combinations appear to exhibit any crystallization behavior. The glass transition temperature of the polymers is slightly below the DMA results, as expected, and no other transitions were revealed in the virgin samples. Figure 11 shows several DSC experiments conducted on samples of PEGDA MW 700 with 3MOPA in order to further understand the increase in modulus seen in the DMA experiments in Figure 9. Each of

these samples was exposed to different conditions chosen based on conditions which could affect the materials behavior during DMA: (a) material which had been compressed by about 10 % while being submerged in water for approximately 12 hours, (b) material which had been compressed by a c-clamp while submerged in water for 24 hours, (c) material that had been submerged and cyclically compressed at a compression frequency of 1 Hz and amplitude of 10%, (d) material that had been submerged and cyclically compressed at a compression frequency of 5.56×10^{-4} Hz and amplitude of 10%, (e) material which had been compressed for 24 hours without being submerged, and (f) material which was neither exposed to water nor stress similar to the material in Figure 10. Materials tested which had been held under compression while being submerged appear to exhibit crystallization and melt behavior as well as the materials that are cycled at a high frequency. Materials that were exposed to compressive stress without submersion or those which were cycled at a low frequency appear to have curves similar to the virgin material that had not been exposed to any stress or subjected to water degradation. The DMA cycles the material under high frequency and the material is stored in and tested in a humid environment, and the DSC testing implies that these conditions can induce crystallization to the material.

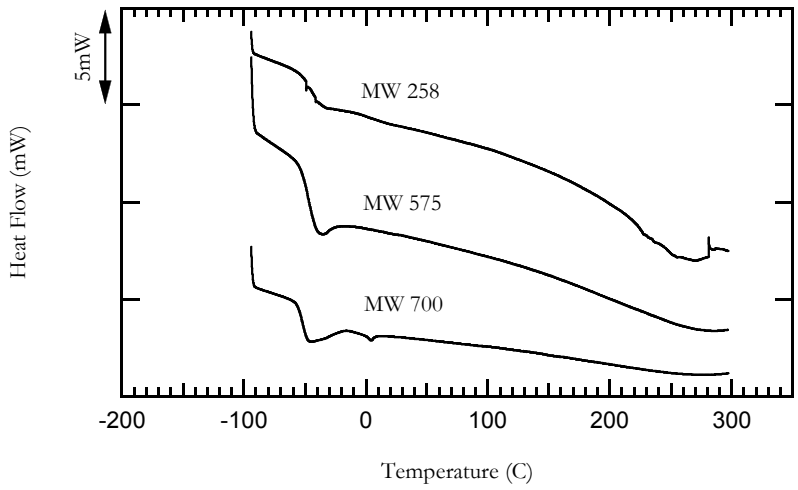


Figure 10. DSC Results for samples of unstrained PEGDA with 3MOPA Networks.

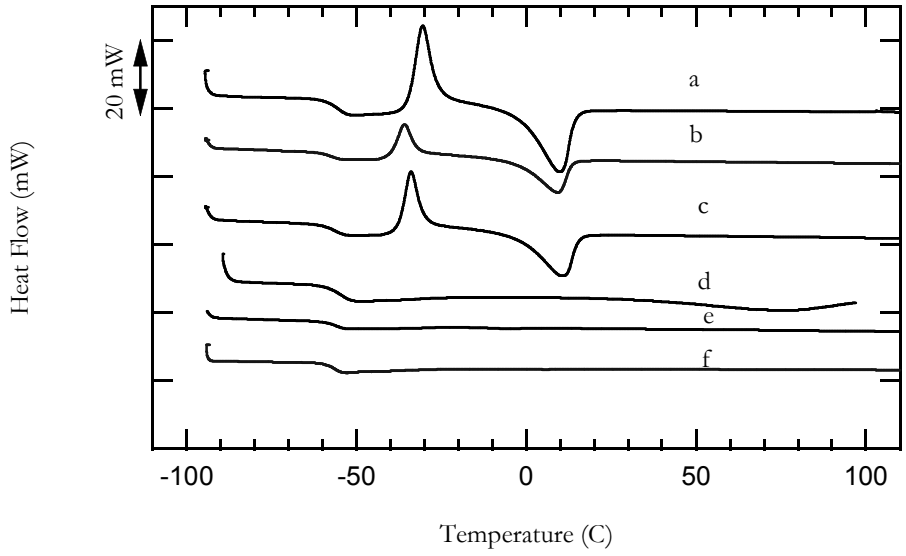


Figure 11. DSC results displaying the crystallization behavior of PEGDA MW 700 with 3MOPA.

Degradation Behavior

After characterization of the three combinations of PEGDA with 3MOPA using the above mentioned methods, tracking of the degradation behavior was carried out on PEGDA MW 700 with 3MOPA, which was primarily chosen because of its degradation products at its relatively rapid rate of degradation. Prior work on 0.8 cm x 1.2 cm x 1mm material samples experienced 24 hour degradation when exposed to a PBS bath on an orbital shaker at 37˚C with a pre-degradation modulus established by nano-indentation of 10 MPa[5]. Figure 12 shows the percent weight loss from cylindrical samples (24 mm in diameter and 12 mm in height) calculated every 2 hours during soaking in PBS for 24 hours at 37 ˚C. As expected, the material uptakes water in the first four hours, causing a slight weight gain followed by a steady weight drop. The degradation of this material results in a significant weight loss (50%) in a 24-hour period for our samples, with

enough material remaining intact so that the material could undergo further examination. Over the 24-hour period, the samples weight loss is gradual without any significant bursts in weight loss rate.

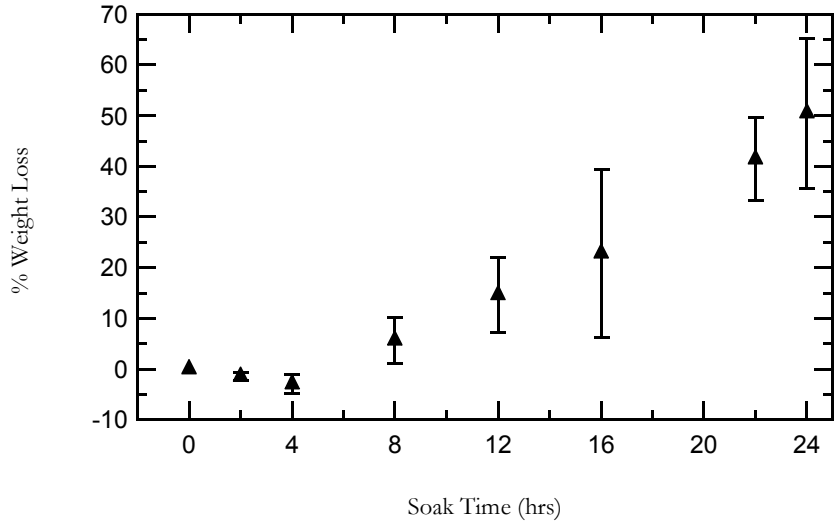


Figure 12. Percent Weight Loss versus Time of Sample in PBS for PEGDA MW 700 and 3MOPA Network.

In parallel with weight loss studies, a non-cyclic baseline for all modulus tests was established by soaking samples of the material in PBS at 37˚C for varied time periods followed by drying the material for 48 hours prior to modulus measurement, similar to conventional testing techniques on biodegradable polymers. Figure 13 shows the modulus of the material as influenced by hydrolytic degradation alone. The modulus at time zero is approximately 2 MPa. We also see that unlike the results for weight loss versus soak time seen in Figure 12, in which the weight loss is really not measurable until the 4th hour due to water uptake, a decrease in modulus is seen within the first 2 hours of soaking. Also unlike the weight loss versus soak time curve, which has a steady positive slope the slope of the modulus versus soak time curve is more severe in the first 12 hours and the begins to level off, although there is still no drastic drop in modulus with degradation time as is sometimes observed in polymers experience dominant bulk degradation.

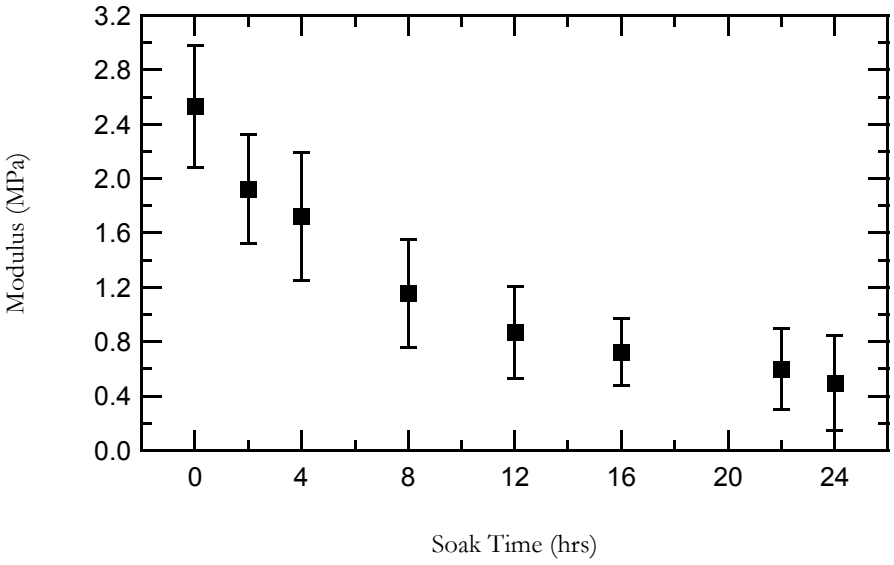


Figure 13. Modulus versus Time of Sample in PBS for PEGDA MW 700 with 3MOPA Network.

Influence of Cyclic Loading on Modulus

In situ Cyclic Testing

Figures 14 -19 show the effects of the compression frequency on the modulus of the material versus the initial baseline modulus established without cycling. Figure 14 shows a cycling frequency of 5.56×10^{-4} Hz versus the baseline. The difference between the modulus of the material for cycling at this frequency versus the baseline is minimal and not statistically significant. The only real difference that is seen between these two cases is that the cycled material had failed (fractured into multiple pieces) after 18 hours. This result indicates that modulus drop alone may not be good parameter for characterizing the performance of a load bearing biodegradable material. Figure 15 similarly shows material cycled at a frequency of 2.78×10^{-4} Hz versus the baseline. Again there is no statistical difference between the modulus of the cycled material and the baseline material, but now the cycled material fractures after 22 hours. In Figures 16 and 17 the material was cycled ever 15 minutes and every 5 minutes respectively, and in

both cases fractures after 23 hours. In both cycling tests in Figure 16 and 17 the modulus of the cycled materials decreases slower that that of the baseline material. This result is observed more so in the 5 minute cycled samples than the 15 minute cycled samples, although the trends are not statistically significant. The final figure, Figure 18 shows the results for a material that is cycled every 1 second versus the baseline. Although the experimental scatter is quite large the average modulus drop is consistently less than that of the baseline material, and the material fails after 20 hours of cycling in water after modulus has dropped less than 50%.

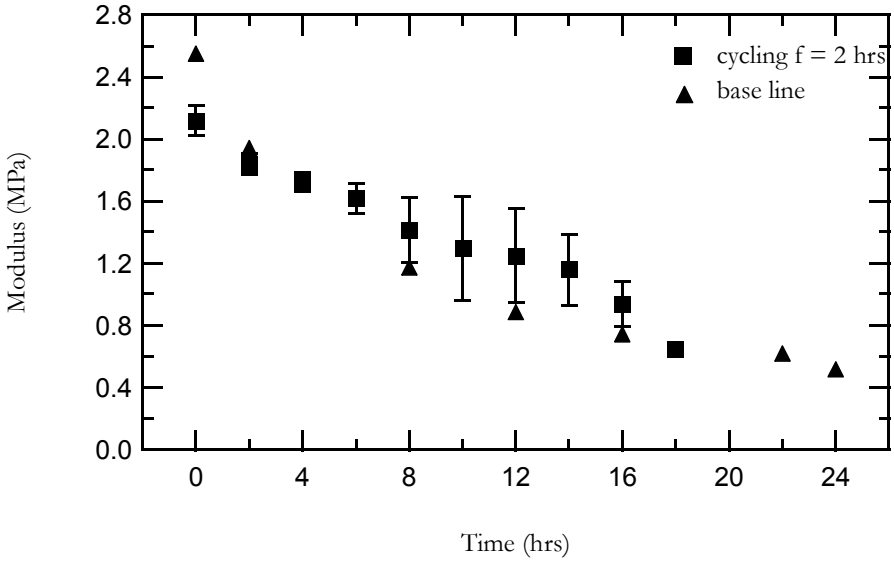


Figure 14. PEGDA MW 700 with 3MOPA networked samples cyclically loaded every 2 hour until failure.

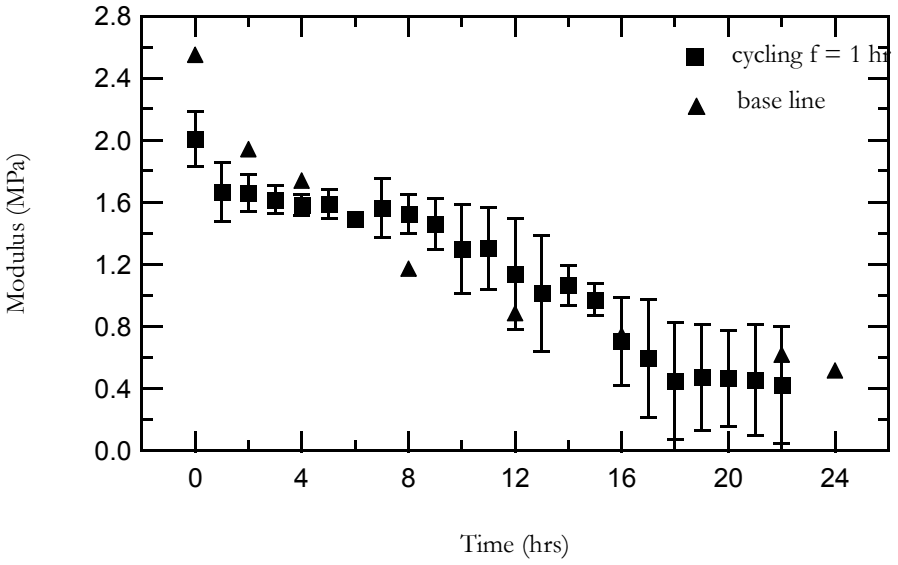


Figure 15. PEGDA MW 700 with 3MOPA networked samples cyclically loaded every hour until failure.

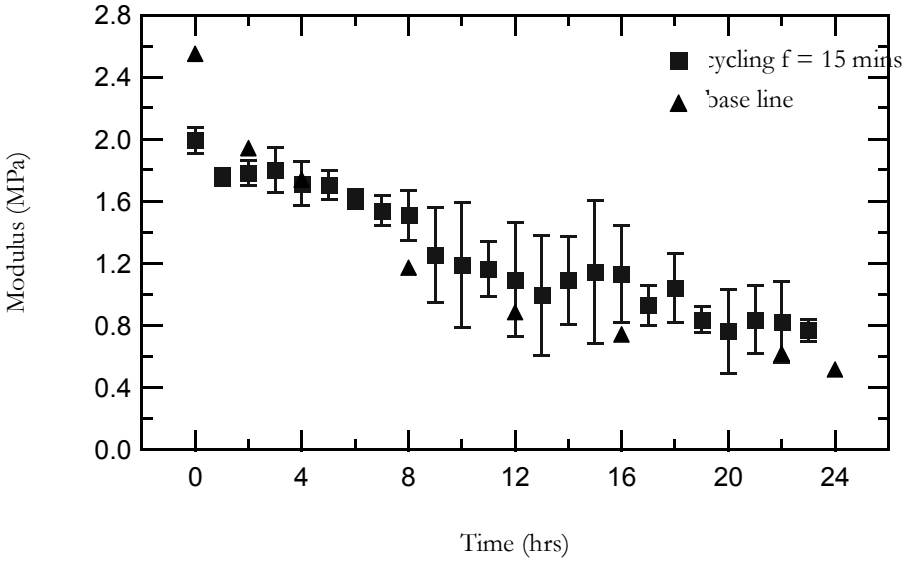


Figure 16. PEGDA MW 700 with 3MOPA networked samples cyclically loaded every 15 minutes until failure.

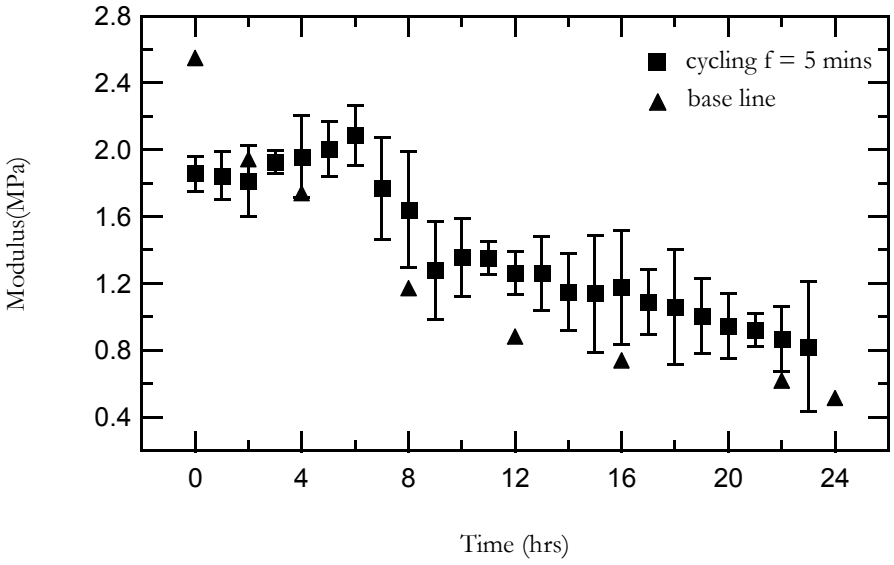


Figure 17. PEGDA MW 700 with 3MOPA networked samples cyclically loaded every 5 minutes until failure.

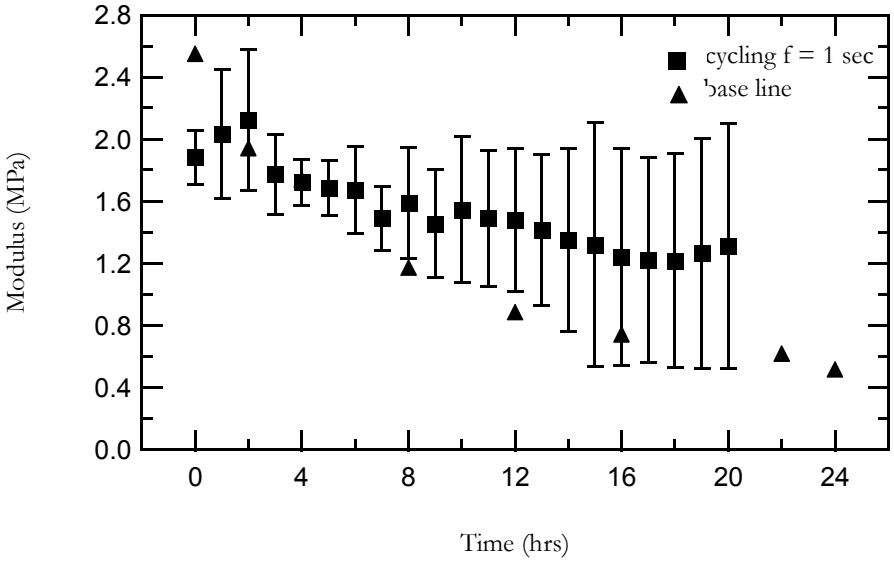


Figure 18. PEGDA MW 700 with 3MOPA networked samples cyclically loaded every second until failure.

Figure 19 compares average modulus versus time curves for all cycling frequencies and also shows the in situ cycled results as compared with a dry sample cycled at a frequency of 1 Hz. Figure 15 demonstrates a slight, but statistically insignificant, trend of decreasing degradation rate with increasing cyclic frequency, and

also demonstrates that 1 Hz cycling alone is unable to result in a change in modulus over the timeframe of the experiment. This baseline data, along with the change in degradation rate and early failure of the cycled samples demonstrates the interaction effect that may occur between mechanical cycling and hydrolytic degradation. Future work at various cyclic strain amplitudes should be considered to elucidate this effect.

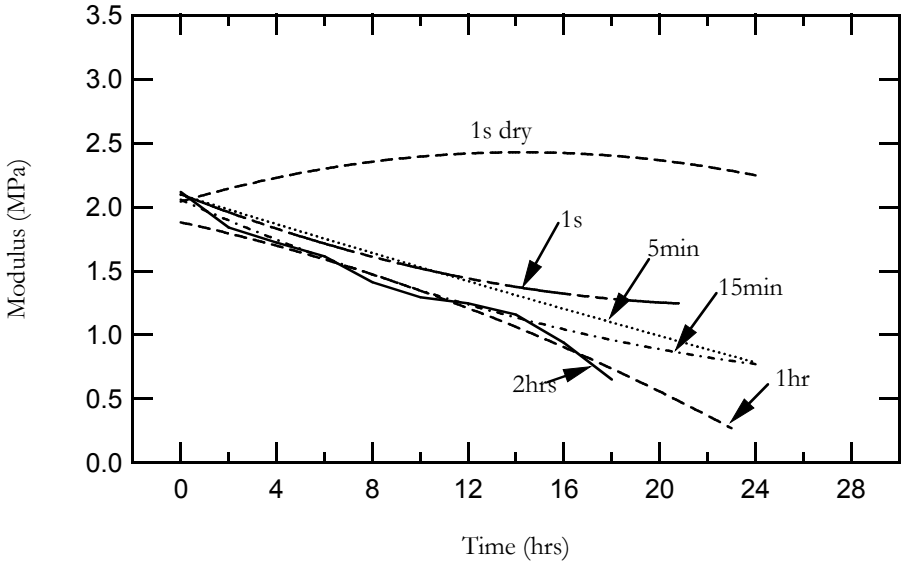


Figure 19. Comparison of the modulus for PEGDA700/3MOPA as the sample degrades during in-situ cyclic testing where compression occurs every 2hrs, 1hr, 15mins, 5mins, and 1 s.

CHAPTER 4

DISCUSSION

Although the synthesis of various poly(beta-amino esters) has been previously established, relatively little is known regarding their structure, thermo-mechanical properties, and degradation behavior under mechanical loading. The intent of this study was to better understand the synthesis, structure, and mechanical properties of a specific set of poly(beta-amino esters) during degradation. In addition to baseline and cyclic loading influenced degradation experiments we performed basic studies to understand the structure of the material including ^{1}H NMR to track the formation of the macromer mixture, PhotoDSC to characterize network formation, DMA to establish the glass transition and rubbery modulus (cross link density), and DSC to understand crystallization behavior.

Reaction and Network Characterization

^{1}H NMR

The ^{1}H NMR experiments allowed us to observe the disappearance of the peak associated with the primary amine group[19] labeled as k, which can be seen quite vividly in Figure 5. This peak disappears immediately, signifying the step of the primary amine converting to a tertiary amine within the first six hours of the reaction. Two peak formations, labeled l and m in Figure 4, also are observed after 24 hours of mixing of the two monomers and the progression of their formation can be seen in Figures 5 and 7. These peaks are related to hydrogen groups attached to carbons which were previously part of the acrylated end and now form a new bond with the amine.[19] Another significant finding of the ^{1}H NMR is the disappearance followed the reappearance of the peak labeled e. This peak is associated with the hydrogen in the long PEG back bone.[19] The

disappearance during early mixing may be related to an intermediate in the bonding with the amine. It is believed that this intermediate may not exhibit the symmetry require for this peak to appear, although this has not been confirmed.

PhotoDSC

The formation of the crosslinked networks in PEGDA 258, 575 and 700 with 3MOPA occur due to simultaneous polymerization and crosslinking of multifunctional macromers. For all conditions present in the PhotoDSC experiments, it was shown that the larger the molecular weight of the PEG unit in the macromer the quicker the network conversion time. Increasing the molecular weight of PEG chains statistically lengthens the macromer for equivalent reaction kinetics and thus decreases the total fraction of active acrylate bonds relative to inactive molecular weight. Longer macromers, with less total reactive sites would be expected to complete polymerization more rapidly given equivalency of propagation kinetics during network formation. This hypothesis has not been proven there, and other effect may be playing a role. For example, the shorter PEGDA molecules may form molecules with less ability to diffuse and coil. Decreased ability to diffuse and accommodate polymerization, would slow down the kinetics of conversion in the networks for the macromers build from the shorter PEG chains.

DMA

The modulus-temperature response and glass transition of the materials provides key structural information on the polymers. We will first discuss the rubbery modulus of the material followed by glass transition behavior. Based on rubbery elasticity, the rubbery modulus of the material is proportional to crosslinking density. From the DMA experiments shown in Figure 9 the rubbery modulus of PEGDA 575 with 3MOPA is largest of all the combinations followed by PEGDA 700 and then PEGDA 258. This result implies that effective cross link density is lowest in the PEGDA 258 material and

highest in the PEGDA 575 material. These trends are somewhat counterintuitive, and some explanation can be provided. First, the incomplete crystallization in the PEGDA 700 material (notice its rubbery plateau continues at higher temperature) results in an "effective" increase in crosslink density that is not linked to the chemical network. In fact, rubbery modulus measurements made through non-cyclic mechanical testing reveal a modulus in the range of 2 MPa, a value that puts the PEGDA 700 material at the bottom end of the modulus scale. The difference between the PEGDA 575 and PEGDA 258 is more difficult to understand. The higher modulus in the PEGDA 575 material implies that either (a) the PEGDA 575 material created the shortest diacrylate macromer chains, resulting in the higher cross-link density or (b) the chains were of similar length and the material containing PEGDA 258 material chains hindered network formation due to their conformability. In future work, molecular weight measurements on the macromers should be performed to sort out the two possibilities.

The PEGDA 575 with 3MOPA had the highest T_g at -31˚C, followed by PEGDA 258 with 3MOPA at -35˚C, and the lowest T_g at -39˚C was measured in PEGDA 700 with 3MOPA. The low transition temperature in the PEGDA 700 is expected based on the long length of the flexible, inherently low T_g, PEG units. In addition, this material, prior to crystallization, likely has the longest distance between crosslinks, which also serves to maintain a lower T_g. The T_g of the PEGDA 258 mixtures occurs between the T_g of the PEGDA 700 and PEGDA MW 575 mixtures. The reason for this can be attributed fully to the crosslink density in this network. The high, and broader, T_g of the PEGDA 575 is likely due to a higher crosslinking density, consistent with the higher rubbery modulus value. Finally, although the variation in T_g between the three materials is statistically significant, the difference is small and likely inconsequential for application. The appearance of peak in the modulus of the material following the glass transition in the DMA results for PEGDA 700 with 3MOPA is intriguing. It is well known that the uniaxial stress in semi-ordered networks can lead to crystallization[20]. During DMA,

1mm thick samples are placed in tensile grips and the strain is oscillated sinusoidally in one direction, which could lead to crystallization. Immediately following the increase in modulus there is a decrease and then the modulus begins to climb slightly again representing elastomeric properties one would assume to be present in such a crosslinked network. This information indicates that only a small fraction of the material crystallized. Further experiments were done using DSC to determine the factors driving crystallization.

DSC

DSC of the unstrained samples showed no transitions aside from the glass transition. First, a material was strained under compression for 24 hours and then tested in the DSC revealing only the glass transition. Since, water is a known solvent of PEGDA MW 700 and 3MOPA networks; we explored two crystallization hypotheses. The first idea was that the intake of water into the voids in the strained material forms bridges between the chains and therefore crystallinity[21]. This likely explains the crystallization and subsequent melt that is seen in the DMA results because the samples exposure to water is minimal. The second theory, which is of greater interest and importance as it is more likely to be a problem during application of this material, stems from the degradation of the material which could cause the breaking of bonds and under strained conditions those bonds could reorient and form new bonds causing crystallization. In order to test this theory, samples of material were strained by compression in water and in air using a c-clamp and also using the Insight™ mechanical tester. The samples strained in the presence of water were compressed 12 -24 hours which would allow the samples ample time to get passed the swelling phase and begin degrading. Afterwards, those samples were tested on the DSC. These samples demonstrated crystallization and melt behavior. Since crystallization may impact the cyclic degradation tests, we tested some materials which were cycled during degradation

on the DSC. Samples were submerged and cycled for 24 hours at a frequency of 5.56 x 10^{-4} Hz and a frequency of 1 Hz prior to testing by DSC. The results showed that crystallization occurred only during the high frequency cycling.

DSC was performed to give more insight as to what transitions, aside from the glass transition, could have been occurring in these samples. DSC of the unstrained samples showed no transitions aside from the glass transition. During the DMA experiments an increase and decrease in modulus was observed as a result of crystallization and melt occurring in the part of the material. Therefore, material was taken and strained by compression for 24 hours and then tested in the DSC. Again, no transitions aside from glass transition were observed in the DSC data. It has been observed that a small amount of condensation accumulates in the sample chamber of the DMA during testing. Since, water is a known solvent of PEGDA MW 700 and 3MOPA networks; there are two possible theories to test. First, exposure to water initially causes the networks to swell. It is believed that the swelling of the material causes some of the material on the surface of the network to re-orient and crystallize[22]. This likely explains the crystallization and melt that is seen in the DMA results. The second theory, which is of greater interest and importance as it is more likely to be a problem during application of this material, stems from the degradation of the material which could cause the breaking of bonds and under strained conditions those bonds could reorient and form new bonds causing crystallization. In order to test this theory, samples of material were strained by compression in water and in air using a c-clamp and also using the Insight™ mechanical tester. The samples strained in the presence of water were compressed 12 -24 hours which would allow the samples ample time to get passed the swelling phase and begin degrading. Afterwards, those samples were tested on the DSC. These samples did show crystallization and melt behavior. The intent of the study was to perform in situ cyclic testing on the material and at this point, knowing that stress on the samples may cause crystallization which would influence the results we see later, we decided to test

some materials which had be cycled during degradation on the DSC. Samples were submerged and cycled for 24 hours at a frequency of 2 hours and at a frequency of 1 second followed by testing DSC. The results showed that at the high frequency of 1 second the samples did crystallize and at the low frequency they did not.

Degradation and In situ Cyclic Testing

The degradation tests on the networks revealed several interesting findings. Consistent with groundbreaking work on this system, degradation without applied load occurred relatively fast over a 24-hour period. The addition of cyclic loading influenced the degradation rate in a non-statistically significant manner. Nevertheless, there was a slight decrease in degradation rate with increasing frequency of compressive cycling which is believed to be influenced by a decrease in diffusivity of water under compression and crystallization. These driving influences would need further exploration. However, the cyclic loading introduced a premature fracture of the samples during testing. This effect was statistically significant, and is important to consider in future testing of biodegradable polymers considered for load bearing applications. Moreover, although the samples demonstrated only a small change in degradation rate with application of 10% compressive cyclic loading, the application of larger cyclic strain levels, or cyclic tensile strains, could completely change the impact of cycling on degradation. Future work is recommended in this area for this system and other biodegradable polymers. It would be important to characterize and understand changes in drug release rate, rate of mechanical property drop, and failure mode with the introduction of cyclic loading.

CHAPTER 5

CONCLUSION

The synthesis of poly(beta-amino esters) is straightforward and versatile using a myriad of commercially available monomers, making them a potential platform for various biomedical applications requiring controlled degradation rates, tuned mechanical properties, and selective degradation products. The polymer network formed by co-polymerized 3-methoxypropylamine and poly(ethylene)glycol diacrylate is degradable in water in approximately 24-hours. The molecular weight of the poly(ethylene)glycol diacrylate unit influences both glass transition temperature and rubbery modulus of the biodegradable polymer network.

When co-polymerized with 3-methoxypropylamine and longer poly(ethylene)glycol diacrylate chains can result in crystallization during loading and exposure to humid environments. Applied cyclic compression stress with a 10% amplitude has a small and statistically insignificant influence on degradation rate measured through change in elastic modulus. Applied cyclic compression stress with a 10% amplitude resulted in premature fracture of the biodegradable polymer prior to full degradation.

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